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(54) Title: METHOD OF PREPARATION AND PHARMACEUTICAL COMPOSITION OF AN UNCARIA WATER SOLUBLE EXTRACT (57) Abstract The present invention is directed to a method of preparation and the composition of a water soluble extract of the plant species <i>Uncaria</i> . The present invention is also directed to the pharmaceutical use of the composition for the enhancement of the immune, anti-inflammatory, anti-tumor and DNA repair processes of warm blooded animals. The present preparation of the water soluble extract of the plant species <i>Uncaria</i> results in the depletion of many of the ingredients which lead to various toxic side effects associated with other extracts or compositions derived from <i>Uncaria</i> . Also, the present preparation leads to the depletion of many of the active ingredients commonly associated with other extracts and compositions of the plant species <i>Uncaria</i> . Therefore, the present invention teaches that the hot water extraction of the crude plant parts of <i>Uncaria</i> and the subsequent dialysis of the solubilized products yields a low molecular weight composition which maintains a high degree of the anti-tumor, inflammatory and immune stimulatory activities associated with the crude plant parts.		

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METHOD OF PREPARATION AND PHARMACEUTICAL COMPOSITION OF AN UNCARIA WATER SOLUBLE EXTRACT

5 BACKGROUND AND FIELD OF THE INVENTION

Field of the Invention

The present invention deals with defining a phytomedicinal water extract preparation of the plant species *Uncaria*. In this preparation, many of the ingredients leading to non-specific toxic side effects including palability
10 generated from the presence of such ubiquitous plant toxic compounds as polysacharrides and tannins are depleted (Cragg et al, Amer. Chem. Soc. Symposium Series 534 :81-96, 1993). In addition, the most common biologically active plant products (e.g. steroids and alkaloids) are also depleted. This is accomplished by hot water extraction of the crude plant
15 parts of *Uncaria* and subsequent dialysis of the solubilized products where a high degree of anti-tumor and immune stimulatory activities are maintained in the final dialyzed (low molecular weight) fraction.

Discussion of Related Art

- Drugs derived from higher plants represent about 25% of all prescription drugs dispensed by pharmacies in the United States. For example, over 35,000 plant species have been screened between 1960 and 1986 for cytotoxic and antitumor properties which adds additional support to the concept that plant extracts are a potential rich source of medicines (Balandrin et al, Amer.Chem. Soc. Symposium Series 534:3-11, 1993). Most of the clinically useful plant products discovered so far have either been phytomedicines defined as ingested crude plant parts or as plant extract/tincture preparations (e.g. Gingo, Echinacea, Chamomile, St. John's Wort, Saw Palmetto, Hawthorn, Lemon Balm), or as isolated chemical entities of two major chemical categories - namely the steroids and alkaloids (Balandrin et al, Amer. Chem. Soc. Symposium Series 534:3-11, 1993; V.E. Tyler, Amer.Chem. Soc. Symposium Series 534:24-38,1993).
- There is prior art establishing that extracts of the plant species *Uncaria* contain alkaloids, sterols, and triterpenoids which in turn are known to possess antiviral, anti-inflammatory, anti-mutagenic and anti-tumor (cytotoxic) activities (K.Keplinger, PCT Int. Appl. WO 8210, 130, 1985; Wagner et al, Planta Med. 419-23, Oct 5 1985; Senatore et al Boll.Ital. Biol. Sper. 65(5):517-20, 1989; Aquino et al, J. Nat. Prod. 52(4):679-85, 1989; Aquino et al, J. Nat. Prod. 53(3):559-64, 1990; Aquino et al, J. Nat. Prod. 54(2):453-9, 1991; Rizzi et al, J. Ethanopharmacol. 38(1):63-77, 1993). In fact, the available scientific literature teaches that the medicinal properties of the plant species *Uncaria* are due to the presence of these biologically active ingredients in phytomedicinal preparations of this plant. However, these components of *Uncaria* are usually extracted from the plant parts with

organic solvents because of their poor solubility in water. It follows then that this prior art does not teach one skilled in the art that a low molecular weight, water soluble fraction of Uncaria would have any antitumor or immune stimulatory properties. Therefore, the discovery disclosed in this invention, that there is a high degree of biological activity in the hot water, low molecular weight fraction of Uncaria, is not obvious but novel and proprietary.

Phytomedicinal preparations of Uncaria (also known as Una de Gato and Cat's Claw) have been sold in the United States and other countries for years as a herbal medicine. The basic formulation of these products has been encapsulated pulverized plant parts administered orally as 1-3 grams of crude bark per day. This method of preparation and dosing is a serious deviation from the historical medicinal use of Uncaria species described by the Indians indigenous to the Amazon basin of South America. Native Indians prepare treatments of Uncaria by drinking hot water extracts as a tea (1 cup or about 100 ml containing about 0.1-0.4 grams of crude plant parts per day). There is a 10-20 fold increase in the daily doses offered commercially compared to the practice of historical medicinal use. However, commercial Uncaria preparations rely on an efficient extraction and absorption of the active ingredients under the strong acidic conditions existing in the stomach. In contrast, the historical Uncaria medicinal preparations rely on hot water extracting the active components from the particulate fraction before they are ingested. There is no *a priori* scientific reason to believe that commercial preparations of Uncaria duplicate the historical practice of administering efficacious water-extracted doses to humans. For example, it is not obvious or taught by this prior art that acidic digestion of Uncaria crude plant parts in

the stomach would even approximate the efficiency of hot water extraction. In addition, the hot water insoluble materials left behind when preparing tea extracts which are present in commercial crude plant part preparations, might cause stomach irritation, toxicity or limit the absorption of Uncaria's active ingredients. It is significant that a recent review of the literature has revealed that there has never been a single peer-reviewed scientific article demonstrating any efficacious effects from human oral consumption of crude plant parts of Uncaria.

SUMMARY OF THE INVENTION

10 In one aspect, the present invention teaches that if the plant species of Uncaria are hot water extracted, which has been the practice from historical medicinal use, and then dialyzed to deplete ubiquitous non-specific toxic components and the levels of previously identified lipophilic components such as sterols and alkaloids possessing anti-tumor and anti-inflammatory properties, there still remains in the dialyzable fraction a novel
15 phytomedicinal preparation of Uncaria having potent anti-tumor and immune stimulatory properties without any measurable toxic side effects. Any potential medicinal properties possessed by this subfractioned formulation of Uncaria would likely have been depleted of many of the known active
20 ingredients of Uncaria, namely the sterols and alkaloids, because it is a low molecular weight water soluble preparation.

In another aspect, this invention discloses the method by which warm blooded animals could be treated successfully by oral administration of

Uncaria water extracts. Here, it is disclosed that the water soluble portion of crude Uncaria bark at 3736 μg of crude bark per ml (calculated from 198 μg per milliliter dried water extract of C-Med-100, Fig.1, Example 2 and the yield of C-Med-100 extract from crude bark = 5.3%, Example 1) is necessary
5 in order to inhibit 50% of tumor cell growth in vitro following a single dose. This would translate into a 70 kg person having to take 262 one gram capsules containing crude pulverized bark per day. It follows then that this invention permits the methodological advantage of delivering a safe and more efficacious in vivo treatment of Uncaria at doses 100 times higher than have
10 ever been previously achieved with either commercial or historical preparations. Moreover, being water soluble, the new dialyzable fraction of Uncaria plant parts could be easily dried and combined with non-toxic inert carrier or diluent for convenient oral administration. Examples of such non-toxic, inert carriers include, but are not limited to, wheat starch and sodium
15 carboxymethyl cellulose.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. The effect of C-MED-100 extract (Example 1) on cell proliferation. The left panel shows the growth curves while the right panel shows the regrowth curves. At the last day of the experiment, cells in the
20 groups having C-MED-100 extract at 397 $\mu\text{g}/\text{ml}$ were spun down, counted and resuspended in normal medium or medium with C-MED-100 extract at 397 $\mu\text{g}/\text{ml}$. Cell survival was assessed by trypan blue exclusion.

Figure 2. Time course of apoptosis in HL-60 cells induced by C-MED-100 extract (Example 1) as compared to negative (no treatment) or positive (100 μ M H₂O₂ exposure) controls.

The data displayed in Figure 2 teaches that C-Med-100 extract induces a time
5 dependent increase in apoptotic cells over the entire evaluation period of 72
hours. Hydrogen peroxide, which was used as a positive control, caused
massive cell death by apoptosis within 24 hours, after which time no more
apoptosis occurred. This difference in response over time between C-Med-
100 extract, and the classic apoptotic agents represented here by hydrogen
10 peroxide, supports the hypothesis that the mode of action of induction of
apoptosis by C-Med-100 extract is unique. In this regard, it offers the
advantage of developing a phytomedicine having a selective mode of
inducing cytotoxicity by apoptosis that, in turn, influences the progression of
disorders such as inflammation, cancer and immunosuppression. In addition,
15 these studies were performed on human leukemic cells (HL-60) which also
directly demonstrates the ability of C-Med-100 extract to kill tumor cells.

Figure 3. Dose response of acidic preparations of crude bark and C-MED-
100 extract (Example 1) of Uncaria to inhibit the growth of leukemic HL-60
cells. Cell proliferation was assessed by the [³H]thymidine incorporation into
20 DNA after 3 days of culture and 1 hour labelling. The data points represent
the average of 3 samples and expressed as percentage of controls.

The data reported in Figure 3 show that the IC₅₀ values (i.e. the dose
inducing 50% inhibition of cell proliferation) for C-Med-100 extract modified
to equal the oral ingestion of an equivalent crude bark preparation of Uncaria

was about three times more biologically active at inhibiting tumor cell growth. This result teaches the superior method of using C-Med-100 extract in preference to the current phytomedicinal preparations for oral ingestion of Uncaria because smaller initial doses of the C-Med-100 extract can be
5 practically employed more effectively to achieve in a single dose of even greater efficacious health benefits such a preventive antitumor treatment.

Figure 4. Body weight change after C-MED-100 extract (Example 1) was supplemented daily to W/Fu rats for 6 weeks.

10 Figure 5. Haemoglobin (HGB) and white blood cell (WBC) counts of blood after 4 weeks of daily supplementation of C-MED-100 extract (Example 1) to W/Fu rats. Blood was sampled from rat orbital vessels in heparinized tube and measured by an automated haematological analyzer. Data shown are average in column and standard deviation (SD) in error bar (n = 8-10). P values shown are by one-tailed t-test.

15 Figure 6. DNA repair enhancement by C-MED-100 extract (Example 1) supplementation in a rat model. DNA damage and repair were measured by alkaline elution of spleen single cell suspensions from female W/Fu rats. C-MED-100 supplemented rats (8 and 16 mg/day for 8 weeks) were irradiated with or without 12 Grays and allowed to repair in vivo for 3 hours. Data
20 shows the averages in column and SD by error bar (n>=5 in each group).

Figure 7. Phytohemagglutinin (PHA) stimulated lymphocyte proliferation in W/Fu female rats supplemented with C-MED-100 extract (Example 1) for 8 and 16 mg/day for 8 consecutive weeks. Splenocytes were prepared by

single cell suspension (Olsson et al., 1995, Carcinogenesis 16(5): 1029-1035) and cultured on a microliter plate at 25,000 cells/well in 200 μ l RPMI 1640-10% fetal calf serum-10 μ l PHA at 37 C, 5% CO₂ for 5 days, then pulsed for 6 hours with 0.5 μ Ci [³H]thymidine/ml. Labeled nuclear material was
5 collected on glass fiber filters in a microliter plate cell harvester, dried and counted in scintillation fluid. The resulting cpm [³H]thymidine data was log-transformed to get a near normal distribution. Results are shown by average in column and SD in error bar. n=5 in each group except (low+high)n=10.

DETAILED DESCRIPTION OF THE INVENTION

10 The following examples are some preferred features but not limitations of this invention.

EXAMPLE 1

Preparation of Uncaria extracts (C-Med-100). One hundred and fifty grams of air-dried bark (collected from Campa Indians by CampaMed, Inc.,
15 Arlington, Vermont) or pulverized bark powder (supplied commercially by MW International, Inc., Hillside, New Jersey) of Uncaria (Willd), also known commonly as Una de Gata or Cat's Claw, were mixed with 5 liters of tap water and heated in a stainless steel pot to the subboiling point (about 90-100°C) for 20-24 hours until the hot water extract was concentrated to about
20 200-1000 milliliters by evaporation. The dark brown extract was then adjusted to exactly 1000 milliliters, filtered through common coffee filters (Melitta Scandinavia AB), and then centrifuged at 3000 X g for 15 minutes

at 40°C to produce a particulate-free water extract equal to 150 grams crude bark per 1000 milliliters or 0.15 grams per milliliter. Next 50 milliliter aliquots of the water extract were transferred into cellulose membrane dialysis tubing (pore size 2.4 nm, exclusion limit $\leq 12\,000$ molecular weight, KEBO Lab) and dialyzed against 1000 milliliters distilled water for 24 hours at 4°C. After dialysis, the high molecular weight fraction (dark brown) retained in the dialysis tubing was discarded and the low molecular fraction (light yellow) that diffused out was concentrated by a water vacuum evaporation at 50°C to equal a volume of 50 milliliters. It is this preparation of a hot water, dialyzable (low molecular weight) extract from Uncaria bark that has been biologically evaluated in this invention and hereby is referred to as C-Med-100 extract.

The C-Med-100 preparation is a pale yellow to light brown clear solution with a slight bitter taste and no odor. UV spectral scanning shows a peak absorption at A_{199nm} . C-Med-100 is stable to heat of subboiling for 24 hours and sterilization by autoclaving (20 minutes at 121°C) and maintains its biological activity for at least 6 months when frozen in liquid form at -20°C. When dried by frozen vacuum evaporation, light brown particles are produced yielding 7.933 ± 0.249 milligrams per milliliter of the hot water, dialyzed extract. Hence, the yield from crude bark was 7.933 mg per milliliter divided by 150 mg per milliliter or 5.3%.

EXAMPLE 2

Anti-tumor activity of Uncaria extract (C-Med-100). The anti-tumor activity of C-Med-100 extract prepared as in Example 1 was evaluated in vitro using

2 human leukemic cell lines (HL-60 and K-562) and a mouse leukemic cell line (Raji). The anti-proliferative potency of C-Med-100 was assessed by counting the total number of cells by microscopic analysis at 400X magnification. The cancer cell lines were seeded in duplicate 2 milliliter cultures at a cell density of 0.5×10^5 cells per culture in 15 milliliter Falcon test tubes. The culture medium was RPMI with 10% fetal calf serum and the test tubes were incubated under standard conditions (i.e. 37°C, 5% CO₂ and 80% humidity). C-Med-100 extract was added at 198 and 397 µg per milliliter together with the cell seeding, and the incubation was continued for 8 days. The total number of cells in each culture was determined every day by hemocytometer counting in the presence of trypan blue. After 8 days of growth assessment, the cultures receiving 397 µg per milliliter of C-Med-100 extract were spun down, the old culture medium discarded and new culture medium added back containing \pm 397 µg per milliliter C-Med-100 extract. This was done to ascertain if the cells surviving 397 µg per milliliter C-Med-100 were actually killed (cytotoxic) or only growth inhibited (cytostatic).

The data presented in Figure 1 clearly shows that C-Med-100 extract has a profound anti-proliferative effect on all three cancer cell lines. Interestingly, K-562 cells, well known to be resistant to induction of apoptosis (D'Amico and McKenna, Radiother. Oncol. 33:3-10, 1994), were also the most resistant to growth inhibition using C-Med-100 extract. This data supports the effects on apoptosis presented in Example 3. Furthermore, the regrowth experiments in Figure 1 indicate that not all phases of the cell cycle of cancer cells are equally susceptible to C-Med-100 extract. It was shown that even when growth was inhibited >95% over an 8 day period in culture, as was the case with HL-60 and Raji cells, when C-Med-100 extract was removed from

the medium at least some cells began to grow after a few more days in culture. This data is consistent with Example 3 and also teaches that some tumor cells must be in a state of growth such that they are resistant to C-Med-100 extract and not capable of undergoing death by apoptosis.

EXAMPLE 3

Induction of apoptosis by Uncaria extracts (C-Med-100). Apoptosis is a natural occurring form of cell death or suicide of particular significance to maintaining competent homeostatic inflammatory and immune responses necessary as a primary defense against many diseases including cancer, viral infections, AIDS, autoimmune and neurodegenerative disorders. Agents that can induce apoptosis are potential anti-inflammatory and anti-tumor drugs because they may have the ability to induce apoptotic death in malignant or inflammatory macrophages or monocytes which are known to be particularly sensitive to induction of apoptosis. Likewise, such agents simultaneously stimulate immune cell function by limiting or reducing the production of TNF α by the inflammatory cells which are a well known natural occurring agent that is cytotoxic to lymphocytes and thus immunosuppressive (Apoptosis reviewed by C.B. Thompson, Science 267:1456-62, 1995). The data in this Example (Figure 2) demonstrates and discloses that C-Med-100 extract of Uncaria described in Example 1 is an effective inducer of apoptosis in HL-60 leukemic cells, and thus this preparation possesses important anti-tumor, anti-inflammatory and immune stimulating properties.

For the purpose of evaluating apoptosis in vitro, human leukemic HL-60 cells were cultured at a density of 0.5×10^6 cells per milliliter in 10% calf serum supplemented RPMI medium in a 5% CO₂ atmosphere at 37°C for 48 hours. The cells were harvested by centrifugation and resuspended in a fresh
5 medium at a concentration of $1-2 \times 10^6$ cells per milliliter in 15 milliliter Falcon test tubes for bioassay purposes. Next the cells were exposed to either 100 µM hydrogen peroxide as a positive control or 397 µg per milliliter C-Med-100 extract prepared according to Example 1 or no exposure for 0-72 hours at 37°C. Samples of cells were taken from the cultures at the
10 time periods indicated in Figure 2 and the % apoptosis in the total cells counted were analyzed and scored by phase contrast morphological criteria.

EXAMPLE 4

Relative cytotoxic dose potency of crude bark versus Uncaria hot water extraction (C-MED-100). Commercial preparations of Uncaria are
15 formulated and sold as crude bark phytomedicines given orally usually in capsules at the dose of 1-3 grams per day. The bioavailability of ingested crude bark has never been determined but necessarily would rely on an efficient extraction and absorption of the active components under the acidic conditions of the stomach. On the other hand, C-Med-100 water extract
20 would not have its bioavailability influenced by the particulate fraction of Uncaria. To estimate the relative bioavailabilities of these two preparations of Uncaria, dose response antiproliferative activities were evaluated against leukemic HL-60 cells.

Crude bark material was first extracted with 1 N HCl for 3 hours at a
25 concentration of 0.15 grams per milliliter which was identical to the concentration of crude bark used to produce C-Med-100 extract. Next, this

acid extract was neutralized with 5 N NaOH, centrifuged at 3000 X g to remove particulate material, and the supernatant (soluble fraction) used for comparison with C-Med-100 extract. C-Med-100 extract prepared as in Example 1 was treated in the same exact way as the acidic crude bark preparation so that they could be compared under controlled extraction procedures for biological activity.

The ability of these two Uncaria preparations to inhibit the proliferation of HL-60 cells was determined using exponentially growing HL-60 cells cultured at the density of 50,000/milliliter in RPMI 1640 medium with 10% fetal calf serum, and \pm mixed with the two different Uncaria preparations in a volume of 50 μ l per 950 μ l of culture to equal the final concentrations for the data points reported in Figure 3 (i.e. 0-600 μ g of the dry weights of the respective acidic aqueous extracts per milliliter). 200 μ l of the \pm treated cells suspensions were seeded in 96-well microliter plates and cultured in an incubator at 37°C with 6% CO₂ and 80% humidity for 3 days. Aliquots of 25 μ l [³H]thymidine (9 μ Ci/milliliter) were added for another 60 minutes before the cells were harvested by vacuum aspiration onto glass fiber filters (Whatman GF/A). While free [³H]thymidine is washed through the filters, the [³H]thymidine incorporated into deoxyribonucleic acid (DNA) is retained. The radioactivity retained on the filters was quantified by liquid scintillation counting.

EXAMPLE 5

In vivo evaluation of toxic side effects and hematologic parameters of Uncaria extract (C-Med-100). This invention discloses not only a unique

composition of, and a preparation for, Uncaria but also that the current phytomedicinal practice is not consistent with achieving efficacious treatments of humans based on the daily doses of crude bark recommended for oral administration. As already pointed out in this invention, to achieve
5 single efficacious doses of Uncaria judged by induction of apoptosis or inhibition of cell proliferation (Examples 2 and 3), at least 262 to 524 grams of crude bark (calculation presented in summary of the invention section) would need to be ingested every day. However, C-Med-100 extract could easily be administered orally at these dose levels in one capsule, tablet or the
10 equivalent. In order to evaluate the toxicological and immunological consequences of dosing C-Med-100 extract prepared as in Example 1 in this concentration range, W/Fu rats initially weighing 150-200 grams were administered daily doses of C-Med-100 extract by oral gavage over an 8 consecutive week period. The rats were kept in ambient temperature of 21°C
15 to 23°C. Lights were kept on from 6 am to 6 pm and the rats were given free access to fresh tap water and standard pellet food and were genetically assigned as a health check. The protocol contained 30 female rats randomly assigned into 3 groups each composed of 10 animals: Group 1 = controls, 1 milliliter sterile water by oral gavage; Group 2 = 8 mg dried weight of C-Med-100 extract per milliliter by oral gavage; Group 3 = 16 mg dried weight
20 of C-Med-100 extract per 2 milliliters by oral gavage. Acute toxicity was monitored every day by the presence or absence of symptoms. Body weight was recorded once per week, and hematological parameters were measured every second week. About 0.3 ml blood sample was taken from the optical
25 venous plexus of the rat into 2 ml polypropylene microcentrifuge tube containing 25 µl 2500 I.E. heparin per milliliter (200 I.E./ml in final concentration) and then immediately analyzed by an automated hematology analyzer (Sysmex, K-1000).

The relevant in vivo data is presented in Figures 4 and 5. No acute symptoms were observed so far in the rats after 6 weeks of daily C-Med-100 oral administration at the 8 or 16 mg daily doses. All the rats gained weight over the experimental period and no statistical difference was found at any of the time points tested among the 3 groups (Figure 4). These data teach that the relatively high doses of C-Med-100 extract, which to our knowledge have never previously been administered in vivo for Uncaria preparations, are safe and free from causing any gross obvious acute or chronic toxic response.

In addition, both the 8 and 16 mg doses of C-Med-100 extract showed a statistically significant increase in hemoglobin ($p < 0.0001$ for both groups by one tailed t-test) and white blood cell counts ($p < 0.05$ and $p < 0.077$ by one tailed t-test) (Figure 5). Increased hemoglobin could be stimulatory to respiration and energy production while elevations in the constitutive WBC could have immune enhancing effects both of which were induced by the C-Med-100 intervention. Hence, the data in Figures 4 and 5, taken together with that reported in Figures 1 and 2, disclose that doses of C-Med-100 inducing favorable in vitro biological responses are also both safe and effective in vivo.

EXAMPLE 6

DNA repair enhancement by C-MED-100 supplementation in rat module.

This example teaches that in vivo supplementation of 8 or 16 mg/day C-MED-100 extract for 8 consecutive weeks in the rat resulted in an enhanced ability to carry out DNA repair and thereby to remove DNA damage that in turn inhibits cell replication and immune function. This data confirms that

C-MED-100 extract supplementation has the ability to stimulate the removal of DNA lesions that can cause human disease such as cancer as well as stimulate immune cell responsiveness.

EXAMPLE 7

- 5 PHA stimulated lymphocyte proliferation in W/Fu female rats supplemented with C-MED-100. This example discloses the immune stimulating properties of C-MED-100 extract supplemented in vivo with 8 or 16 mg/day. Splenocytes from the C-MED-100 treated rats had an enhanced ability to respond to the growth stimulation induced by the mitogen, PHA.
- 10 Various modifications of the methods of preparation, use and composition of the water soluble extract of *Uncaria tomentosa* shown and described herein, will be readily apparent to those skilled in the art from the foregoing descriptions. Such modifications are also intended to fall within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method for preparing a water soluble extract of an *Uncaria* species comprising:

- 5 (a) heating a mixture of water and a substance selected from the group consisting of bark and bark powder of said *Uncaria* species until a brown extract of said mixture is of a desired concentration due to evaporation of said water;
- (b) filtering said brown extract from said mixture;
- 10 (c) centrifuging said brown extract to produce an essentially particulate-free water extract;
- (d) dialyzing said particulate free water extract against distilled water in a dialysis medium such that said water soluble extract of said *Uncaria* species distills out of said dialysis medium and a higher molecular weight brown extract of said *Uncaria* species is
15 retained in said dialysis medium; and
- (e) concentrating said water soluble extract of said *Uncaria* species which distills out of said dialysis tubing by vacuum evaporation.

2. The method according to claim 1 wherein said substance selected from the group consisting of bark and bark powder of said *Uncaria* species is
20 mixed with water at a ratio of 150 grams of bark or bark powder to 5 liters of water.

3. The method according to claim 1 wherein said substance selected from the group consisting of bark and bark powder of said *Uncaria* species is dry.

4. The method according to claim 1 wherein said *Uncaria* species is selected from the group consisting of *tomentosa*, *guianensis*, *pteropoda*, *homomalla*, *perrottetii* and *rhynchopylla*.
5. The method according to claim 1 wherein said water is tap water.
- 5 6. The method according to claim 1 wherein the step of heating takes place at a temperature of 90-100°C for 20-24 hours.
7. The method according to claim 1 wherein the step of centrifuging takes place at 1000-3000 X g for 15-60 minutes.
8. The method according to claim 1 wherein the step of centrifuging
10 produces 0.1-0.2 grams of said particulate free water extract per milliliter of water.
9. The method according to claim 1 wherein the step of dialyzing said particulate free water extract takes place in a cellular membrane dialysis tubing which has an exclusion limit of approximately 12,000 molecular
15 weight or less.
10. The method according to claim 1 wherein the step of dialyzing said particulate free water extract takes place at 4-15°C for 24-48 hours.
11. The method according to claim 1 wherein the step of dialyzing said particulate free water extract against said distilled water takes place at a
20 concentration of 50-100 milliliters of said particulate free water extract against 500-1500 milliliters of said distilled water.

12. The method according to claim 1 wherein the amount of said water soluble extract of said *Uncaria* species concentrated by said vacuum evaporation is equal to the amount of said particulate free water extract dialyzed.

5 13. The method according to claim 1 wherein the step of concentrating said water soluble extract of said *Uncaria* species by said vacuum evaporation takes place at 30-50°C.

14. A water soluble extract of an said *Uncaria* species wherein said extract has the following properties:

- 10 (a) exhibits UV maxima at 199 nm;
- (b) stable to subboiling temperature heat for up to at least 24 hours and sterilization by autoclaving for up to at least 20 minutes at 121°C;
- 15 (c) maintains biological activity for at least 6 months when frozen in liquid form at -20°C; and
- (d) produces a brown particulate yielding 6-9 milligrams per millimeter of said extract when dried by frozen vacuum evaporation.

15. A pharmaceutical composition comprising:

- 20 (a) a pharmaceutically effective amount of the water soluble extract of an *Uncaria* species as claimed in claim 14; and
- (b) a nontoxic inert carrier or diluent.

16. The pharmaceutical composition according to claim 15 wherein said carrier or diluent is selected from the group consisting of wheat starch and sodium carboxy methyl cellulose.
17. The pharmaceutical composition according to claim 15 wherein said
5 pharmaceutically effective amount of said water soluble extract of said Uncaria species is 1-6 grams.
18. A method for enhancing the immune system of a living warm blooded animal, comprising:
- (a) administering a pharmaceutically effective amount of the
10 pharmaceutical composition as claimed in claim 15.
19. The method according to claim 18 wherein the step of administering said pharmaceutical composition is at a dosage such that said warm blooded animal ingests over 1-6 grams of the water soluble extract of an Uncaria species as claimed in claim 14 per day.
- 15 20. The method according to claim 18 wherein said warm blooded animal is a human being.
21. The method according to claim 18 wherein the step of administering said pharmaceutical composition is to treat warm blooded animals afflicted with disorders associated with said immune system.
- 20 22. The method according to claim 18 wherein the step of administering said pharmaceutical composition is to prevent disorders associated with said immune system of warm blooded animals.

23. The method according to claim 18 wherein the step of administering said pharmaceutical composition is orally in a form selected from the group consisting of capsules, tablets, syrups, and liquids.
24. A method for inhibiting the inflammatory response process of a living
5 warm blooded animal comprising:
(a) administering a pharmaceutically effective amount of the pharmaceutical composition as claimed in claim 15.
25. The method according to claim 24 wherein the step of administering said pharmaceutical composition is at a dosage such that said warm blooded
10 animal ingests over 1-6 grams of the water soluble extract of said Uncaria species as claimed in claim 14 per day.
26. The method according to claim 24 wherein said warm blooded animal is a human being.
27. The method according to claim 24 wherein the step of administering
15 said pharmaceutical composition is to treat warm blooded animals afflicted with disorders associated with said inflammatory response process.
28. The method according to claim 24 wherein the step of administering said pharmaceutical composition is to prevent disorders associated with said inflammatory response process of warm blooded animals.

29. The method according to claim 24 wherein the step of administering said pharmaceutical composition is orally in a form selected from the group consisting of capsules, tablets, syrups, and liquids.
30. A method for enhancing the anti-tumor response process of a living warm blooded animal comprising:
- 5 (a) administering a pharmaceutically effective amount of the pharmaceutical composition as claimed in claim 15.
31. The method according to claim 30 wherein the step of administering said pharmaceutical composition is at a dosage such that said warm blooded animal ingests over 1-6 grams of the water soluble extract of said Uncaria species as claimed in claim 14 per day.
- 10 32. The method according to claim 30 wherein said warm blooded animal is a human being.
33. The method according to claim 30 wherein the step of administering is to treat warm blooded animals afflicted with disorders associated with said anti-tumor response process.
- 15 34. The method according to claim 30 wherein the step of administering said pharmaceutical composition is to prevent disorders associated with said anti-tumor response process of warm blooded animals.
- 20 35. The method according to claim 30 wherein the step of administering said pharmaceutical composition is orally in a form selected from the group consisting of capsules, tablets, syrups, and liquids.

36. A method for enhancing the DNA repair process of a living warm blooded animal, comprising:
- (a) administering a pharmaceutically effective amount of the pharmaceutical composition as claimed in claim 15.
- 5 37. The method according to claim 36 wherein the step of administering said pharmaceutical composition is at a dosage such that said warm blooded animal ingests over 1-6 grams of the water soluble extract of said Uncaria species as claimed in claim 14 per day.
38. The method according to claim 36 wherein said warm blooded animal
10 is a human being.
39. The method according to claim 36 wherein the step of administering said pharmaceutical composition is to treat warm blooded animals afflicted with disorders associated with said DNA repair process.
40. The method according to claim 36 wherein the step of administering
15 said pharmaceutical composition is to prevent disorders associated with said DNA repair process of warm blooded animals.
41. The method according to claim 36 wherein the step of administering said pharmaceutical composition is orally in a form selected from the group consisting of capsules, tablets, syrups, and liquids.
- 20 42. The product made by the method according to claim 1.

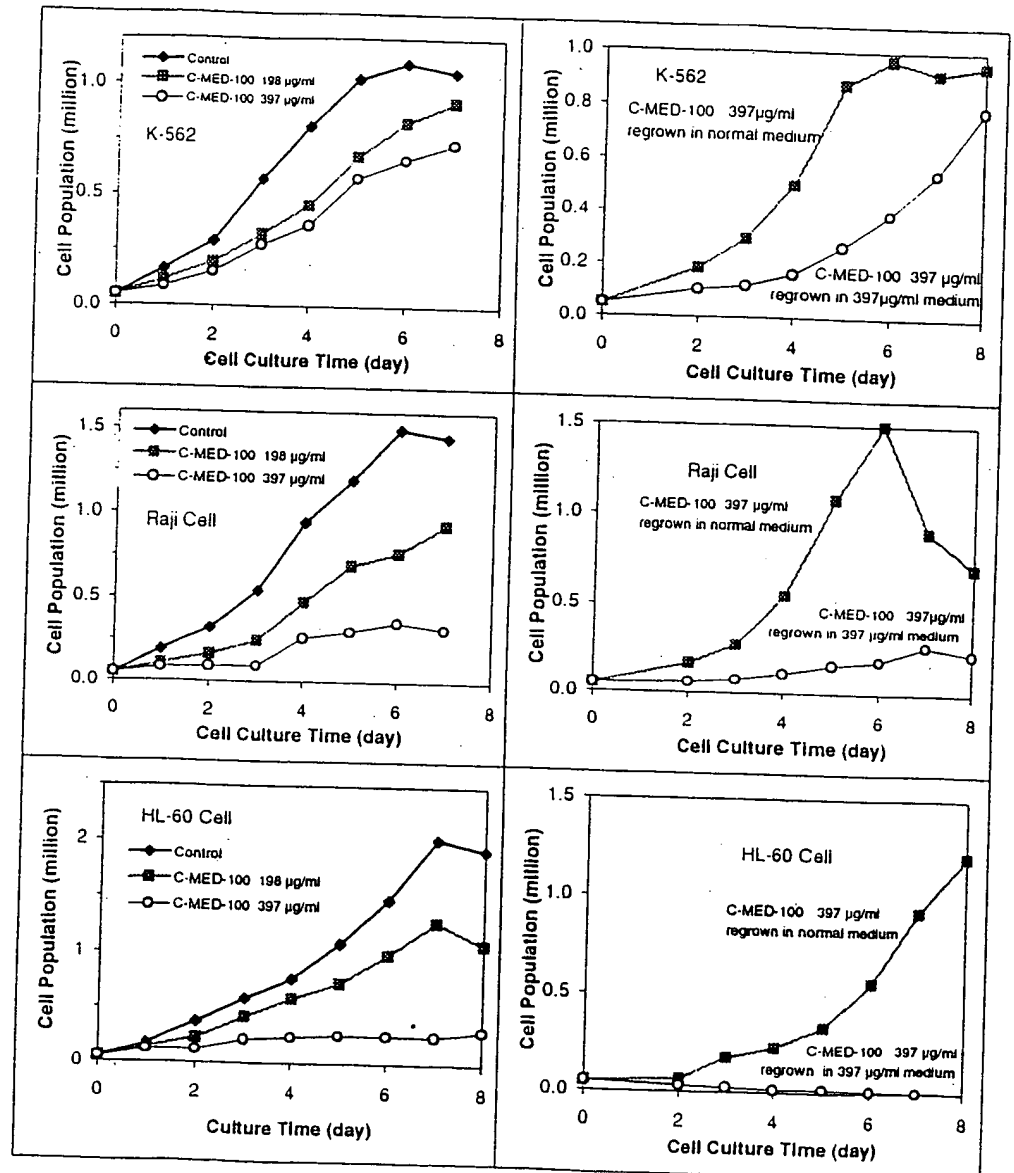


Figure 1

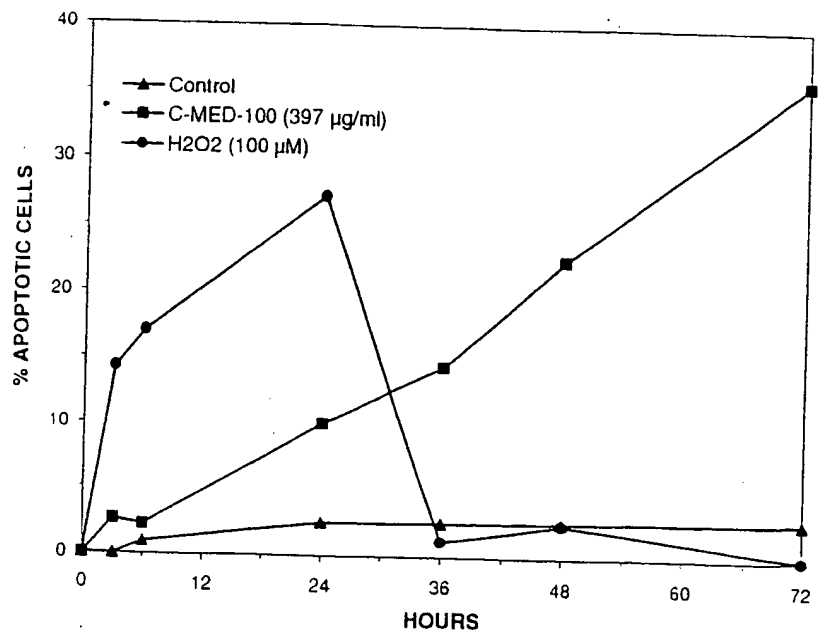


Figure 2

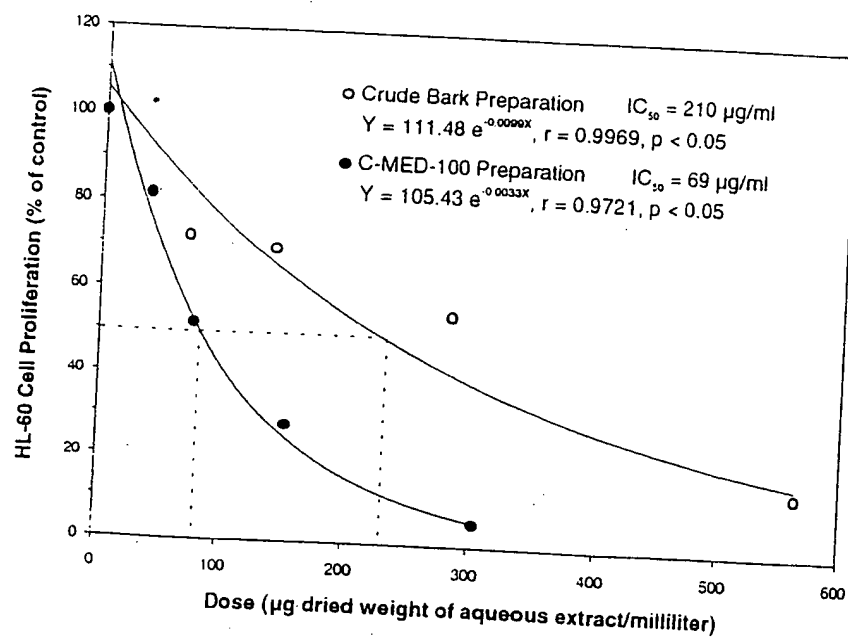


Figure 3

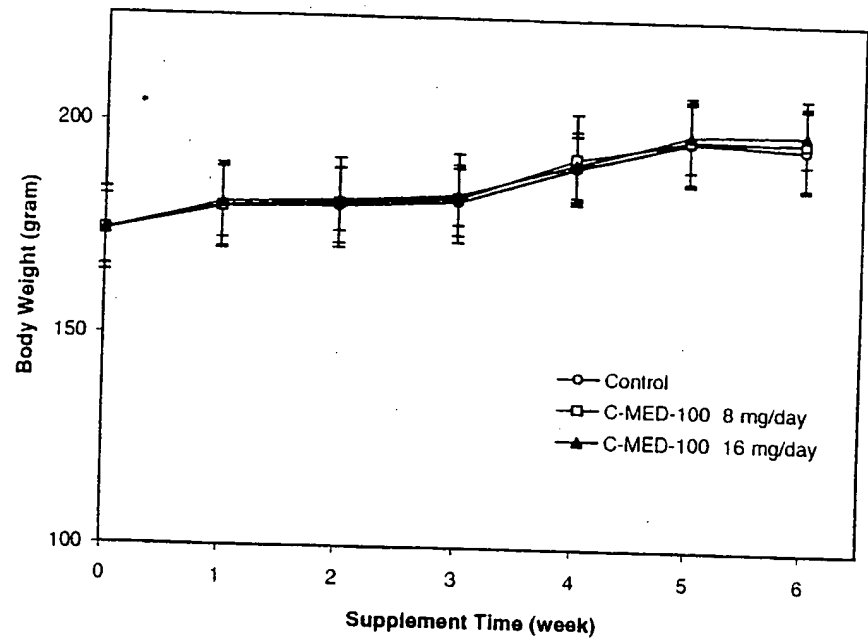


Figure 4

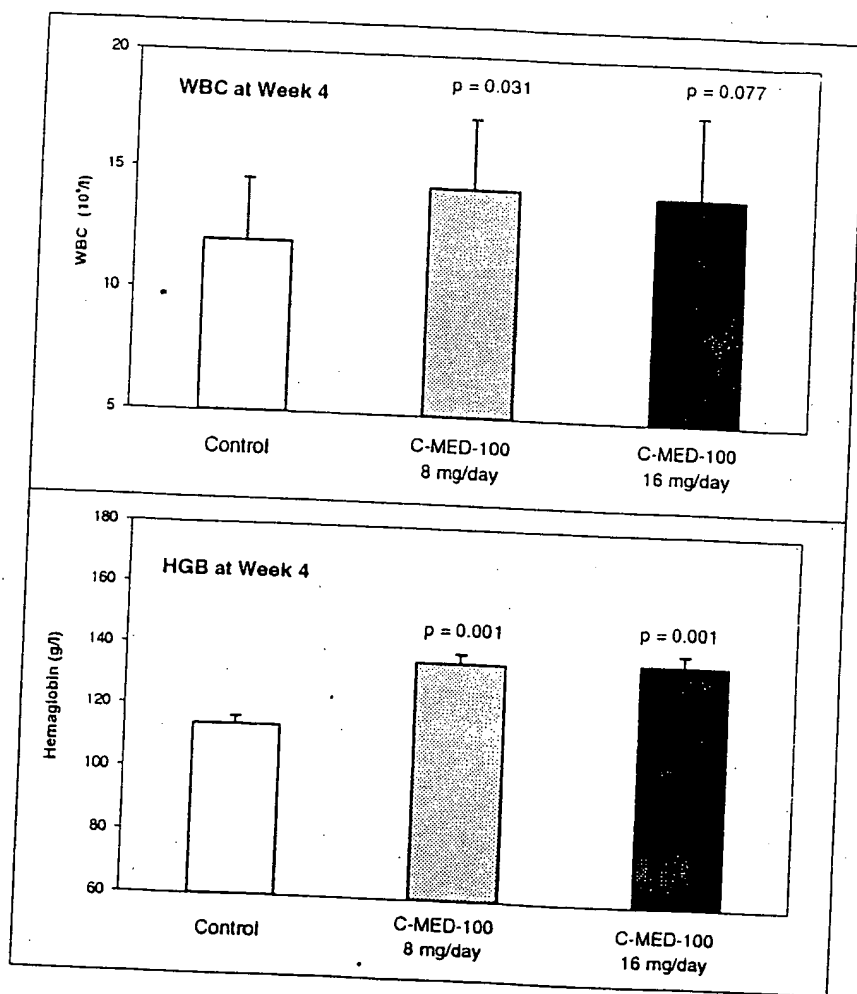
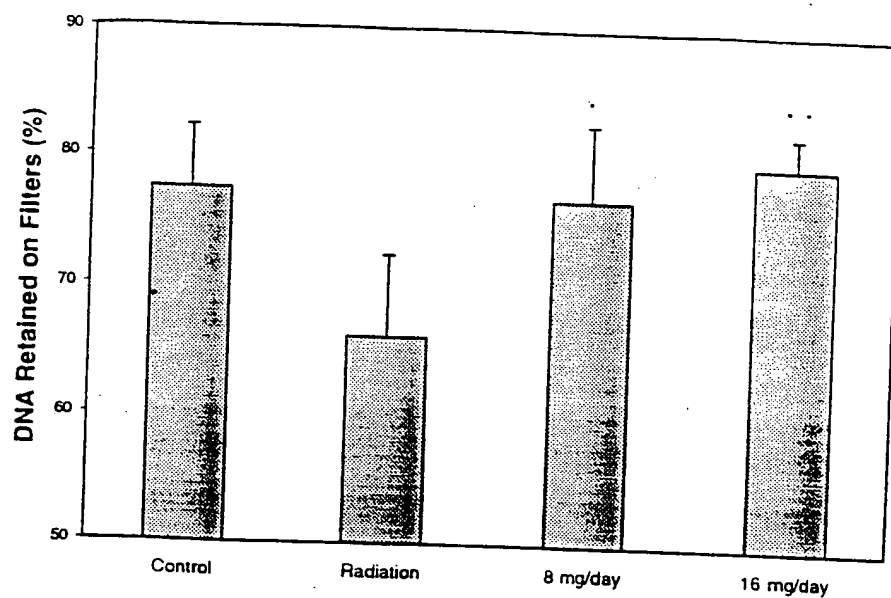


Figure 5



* $p \leq 0.05$ by one-tailed t-test; ** $p < 0.05$ by two-tailed t-test, compared with control group.

Figure 6

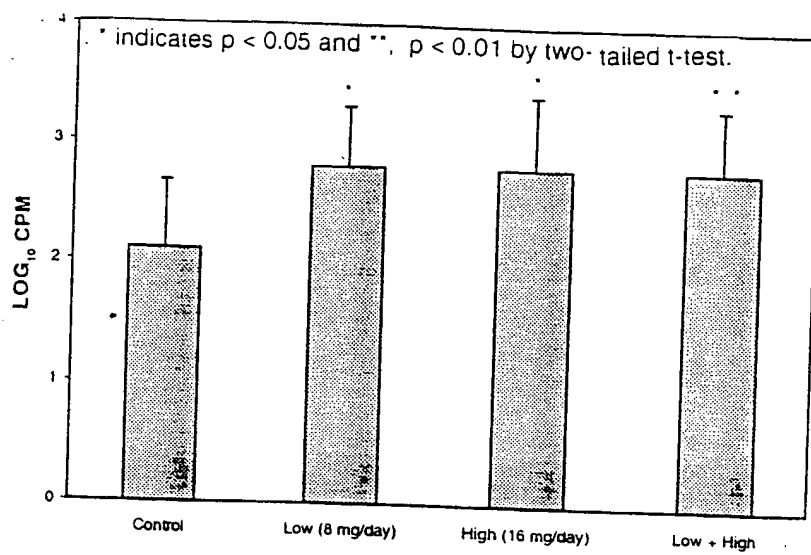


Figure 7

INTERNATIONAL SEARCH REPORT

International Application No
PCT/SE 98/01378

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K35/78

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	REINHARD K -H: "UNCARIA TOMENTOSA (WILLD.) DC. - CAT'S CLAW, UNA DE GATO ODER KATZENKRALLE" ZEITSCHRIFT FUER PHYTOTHERAPIE, vol. 18, no. 2, 15 April 1997, pages 112-121, XP002064093 see the whole document	1-5, 15-41
X	DATABASE WPI Section Ch, Week 9744 Derwent Publications Ltd., London, GB; Class B04, AN 97-478395 XP002096464 & RU 2 076 731 C (YUNONA NAT MED CENTRE) , 10 April 1997 see abstract	1,4, 18-41

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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "S" document member of the same patent family

Date of the actual completion of the international search

12 March 1999

Date of mailing of the international search report

29/03/1999

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Authorized officer

Rempp, G

INTERNATIONAL SEARCH REPORT

International Application No
PCT/SE 98/01378

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE WPI Section Ch, Week 9747 Derwent Publications Ltd., London, GB; Class B04, AN 97-510712 XP002096465 & RU 2 078 578 C (TRESHCHALIN I D) , 10 May 1997 see abstract</p>	1,4, 18-41
X	<p>RAJEEV JAIN ET AL: "ANTI-INFLAMMATORY PRINCIPLES FROM NATURAL SOURCES." HAMDARD MEDICUS, vol. 36, no. 3, 1993, pages 16-27, XP002096463 see the whole document</p>	1,18-41
X	<p>WO 82 01130 A (KEPLINGER K) 15 April 1982 cited in the application see page 2, line 12 - page 4, line 12 see page 13, line 5 - page 15, line 18 see page 17, line 8 - line 14</p>	1-42

INTERNATIONAL SEARCH REPORT

international application No.

PCT/SE 98/01378

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 18-41
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Although claims 18-41 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Claims Nos.: 18-41

Rule 39.1(iv) .PCT - Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/SE 98/01378

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 8201130 A	15-04-1982	AT 13012 T	15-05-1985
		AU 7647581 A	11-05-1982
		EP 0061487 A	06-10-1982
		JP 57501528 T	26-08-1982
		US 4844901 A	04-07-1989
		US 4940725 A	10-07-1990
		US 5302611 A	12-04-1994